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RESEARCH ARTICLE

Pathogenicity of individual isolates of entomopathogenic fungi affects feeding preference of red imported fire ants *Solenopsis invicta*

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Seven isolates of entomopathogenic fungi, *Isaria fumosorosea* (IFCF-H and IFCF-L), *Beauveria bassiana* s.l. (Bb02 and Bb04) and *Metarhizium anisopliae* s.l. (Ma01, SM076 and M09), were selected for their pathogenicity against *Solenopsis invicta* as well as feeding preference of *S. invicta*. When ants were treated with a conidial suspension at a concentration of 1×10^8 conidia/ml, the median lethal times (LT_{50}) of IFCF-H, IFCF-L, Bb02, Bb04, Ma01, SM076 and M09 were 3.4, 162.6, 7.3, 2.8, 3.8, 7.3 and 2.7 days, respectively, after 10 days. The median lethal concentrations (LC_{50}) on the 10th day after inoculation were 1.20×10^7, 1.56×10^{10}, 4.23×10^7, 3.04×10^6, 6.13×10^6, 2.90×10^7 and 9.90×10^5 conidia/ml, respectively. Furthermore, *S. invicta* consumed significantly less solution flavoured with Bb04 conidia than the control, which was demonstrated by the lowest preference index (PREF = 0.09). *S. invicta* did not have a significant feeding preference for other fungal isolates. The pathogenicity (LC_{50}) of fungal isolates was not significantly correlated ($R^2 = 0.013$) with the PREF of *S. invicta*. However, in the paired-choice experiments between different virulent isolates belonging to the same genera, *S. invicta* tended to select the solution flavoured with conidia of relatively lower pathogenic isolates such as IFCF-L, Bb02 and SM076. We conclude that the pathogenicity of congeneric fungi may affect the feeding preference of *S. invicta*. Red imported fire ants might adjust their feeding response to entomopathogenic fungi based on the profile of microbial volatile organic compounds.

Keywords: *Solenopsis invicta*; entomopathogenic fungi; pathogenicity; feeding preference; feeding choice

1. Introduction

Solenopsis invicta Buren, the red imported fire ant (RIFA), is a destructive invasive ant species worldwide. It has been listed as one of the 100 most important quarantine pests (Zhang, Li, Liu, & Zeng, 2007). RIFA invaded Guangdong Province, China, in 2005 and has become a major pest in South China due to its significant direct or indirect effect on human and animal health as well as damage to agriculture and forestry, public facilities and ecosystems (Xu, Huang, Zhou, & Zeng, 2012). Large amounts of traditional pesticides were used in an attempt to eradicate

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RIFA, but these efforts were in vain as the infestation area grew rapidly (Lofgren, 1986). Entomopathogenic fungi are an important aspect of integrated pest management (IPM; Oi & Valles, 2009; Shah & Pell, 2003). Furthermore, the ability of such fungi to self-replicate and disperse within a pest's nest, resulting in an epizootic, makes them promising biological control agents against social insects (Chouvenc & Su, 2012). Three major entomopathogenic fungal genera, *Isaria*, *Beauveria* and *Metarhizium*, have been exhaustively researched and widely applied to control various forest and agricultural pests (Blanford, Jenkins, Read, & Thomas, 2012; Jones, Grace, & Tamashiro, 1996; Ren et al., 2012; Wright & Cornelius, 2012). In the process of pursuing efficacious entomopathogenic fungi to reduce the population of RIFA, Sánchez-Peña and Thorvilson (1992) isolated *Metarhizium anisopliae* from RIFA queens. In Brazil, several *Beauveria bassiana* isolates originated from *S. invicta* were screened for their pathogenicity against RIFA; isolate Bb447 showed great potential efficacy under laboratory conditions and was selected for further development but field application of various Bb447 formulations resulted in the relocation of RIFA (Stimac, Pereira, Alves, & Wood, 1993). The odour of entomopathogenic fungi can be identified by the termite *Coptotermes formosanus*, resulting in enhanced hygienic behaviour, and inducing the failure of an epidemic in the nest (Yanagawa et al., 2011). Hussain,Tian, He, Bland, and Gu (2010) reported that termites elicit stronger electroantennographic responses to *M. anisopliae* than to *I. fumosorosea* and *B. bassiana*. The volatile organic compound (VOC) profile of repellent cultures of *M. anisopliae* is mainly composed of paraffins. Moreover, it appears that virulent fungal strains are more likely to be recognised by social insects and avoided (Mburu et al., 2009). Therefore, screening of entomopathogenic fungi with higher pathogenicity and non-deterrent character simultaneously would be necessary to develop biological control agents to manage RIFA.

RIFA, which is a dietary generalist, relies on opportunistic predation, from sugary liquids to prey protein. Solid foods are usually cut into pieces and retrieved into the nest while liquid foods are placed into reserve in a large and distensible crop. Moreover, in a RIFA colony, protein food is primary allocated to the queens and larvae, because queens need protein to produce eggs while larvae need protein for growth. In contrast, liquid sugar is usually used by the workers to fuel various social activities (Tschinkel, 2006). Variation in the taste response to different foods is a well-known phenomenon in the animal kingdom. However, few studies to date have focused on the impact of a food to which conidia have been added, on the feeding of RIFA.

Although entomopathogenic fungi have been widely used to control many pests in agricultural production, few studies have focused on their use for the control of RIFA in China. Moreover, there is no integrated data about the virulence of fungal isolates while information about the feeding preference of RIFA is unavailable. In this study, we evaluated the virulence of fungal isolates as well as the feeding preference of RIFA for these isolates. The feeding choices of RIFA between congeneric conidial solutions were compared under laboratory conditions. These experiments are necessary for screening appropriate entomopathogenic isolates used for RIFA biological control in the field.

2. Material and methods

2.1. Insects

Colonies of *S. invicta* were collected in Guangzhou, China, and reared in plastic boxes (50 cm × 40 cm × 15 cm) placed in a dark incubator at 25 ± 1°C and 85 ± 1% relative humidity (RH). Colonies were fed *ad libitum* with *Tenebrio molitor* larvae (purchased from the farmers' market) and 25% sucrose water every other day. The worker ants used for experiments were medium-sized workers (*medias*) screened through a combination of 14 and 18 mesh sieves, in which majors were retained on 14 mesh sieves and minors that dropped to the 18 mesh sieve were discarded and only *medias* retained on the 18 mesh sieve were used. The mean ± standard error of the mean (SEM) of *medias*' head width was 1.15 ± 0.1 mm (n = 10) estimated using a microscope with a graticule.

2.2. Fungal isolates and preparation of conidial suspensions

The original hosts and geographical origin of fungal isolates are shown in Table 1. All fungal isolates were cultured in Petri dishes (9 cm diameter) containing potato dextrose agar (per litre: 20 g dextrose, 200 g boiled potato and 20 g agar) and incubated in a constant temperature incubator at 25 ± 1°C, 85 ± 1% RH and 14-h daylight for 10 days. The conidia were brushed from cultures and suspended in 0.01% aqueous Tween-80 and concentrations were measured using a haemocytometer. Conidial preparations were diluted as required for the following bioassays.

Table 1. Detail of fungal isolates used in this study.

Fungal species	Isolates	Original host	Geographical origin	Deposited source	Accession number
I. fumosorosea	IFCF-H	*Coptotermes formosanus* Shiraki	Jiangxi, China	CCTCC	M 2013526
	IFCF-L	*Coptotermes formosanus* Shiraki	Jiangxi, China	SCAU-ERCBC	IFCF-L
Beauveria bassiana s.l.	Bb02	*Ostrinia furnacalis*	Guangdong, China	GDAAS PPRI	Bb02
	Bb04	*Bactrocera dorsalis* Hendel	Guangdong, China	USDA-ARS	03005–C3.10.2
Metarhizium anisopliae s.l.	Ma 01	*Coptotermes formosanus* Shiraki	Jiangxi, China	USDA-ARS	02049–C2.18B
	SM 076	Soil	Guangdong, China	SCAU-ERCBC	SM076
	M 09	Unknown sp.	Australia	SCAU-ERCBC	M09

CTCTCC: China Center for Type Culture Collection; SCAU-ERCBC: Engineering Research Center of Biological Control, South China Agricultural University, China; GDAAS PPRI: Plant Protection Research Institute, Guangdong Academy of Agricultural Science, China. USDA-ARS: United State Department of Agriculture–Agricultural Research Service.

2.3. Pathogenicity bioassay

RIFA workers were collected from the nest and placed in micro-centrifuge tubes containing conidial suspensions at four concentrations: 1×10^5, 1×10^6, 1×10^7 and 1×10^8 conidia/ml. The control group was treated only with 0.01% Tween-80 aqueous solution. RIFAs were submerged in each treatment with gentle swirling for 5 s. Liquid was removed from the surface of RIFA by dabbing on four sheets of filter paper for 10 min. Then the ants were introduced into plastic boxes in which walls were previously coated with Fluon to prevent the escape. They were maintained in continual darkness at 25 ± 1°C and 85 ± 1% RH for 10 days. RIFA mortality was recorded daily and dead ants were removed to prevent any secondary infection. Corpses were surface sterilised and monitored for the appearance of conidia for another 10 days.

2.4. Feeding preference bioassay

The feeding preference of RIFA for entomopathogenic fungi was tested by the food intake rate (%) of the conidial suspensions on filter paper discs by the method modified from Ohmura, Ozaki, and Yamaoka (2006) and Yanagawa, Yokohari, and Shimizu (2010). Briefly, four pieces of filter paper (1 × 1 cm) were placed equidistantly on the bottom of each plastic box. Two diagonal pieces were treated separately with 40 µl of 5×10^7 conidia/ml suspension including 2 mg/ml of brilliant blue (Blue Food Color No. 1, Sigma-Aldrich) suspended in 0.01% Tween-80, while others were treated separately with 40 µl of colourless 0.01% Tween-80 (Treatment I). Treatment II involved experiments set-up to avoid the influence of brilliant blue on RIFA: two diagonal pieces were dampened with brilliant blue suspended in 0.01% Tween-80 while two other pieces were dampened with 40 µl of 5×10^7 conidia/ml suspended in 0.01% Tween-80. Ten *medias* workers were placed in each box and allowed to feed on solutions for 24 h. Each treatment was repeated 10 times within a 24-h period. Thereafter, workers in each box were transferred into a 2-ml tube. Then, 600 µl of 50% EtOH and three glass beads (213–300 µm) were added to each tube. Tubes were loaded into a bead beater (Mini-Bead beater; Biospec Products, ESGC, Switzerland), and samples were homogenised at a high speed (0.43 HP motor) and centrifuged once at 12,000 rpm for 8 min. After centrifugation, the absorbance (Abs) of each supernatant was measured using a UV-Vis spectrophotometer (UV-1800, Shimadzu) at $\lambda = 630$ nm. The olfactory preference index (PREF) for each microbial solution was calculated from the following equation (modified and adapted from multiple sources):

$$\mathrm{PREF} = \mathrm{Abs}_{\mathrm{Treatment\ I}} / \mathrm{Abs}_{\mathrm{Treatment\ II}}$$

In this equation, $\mathrm{Abs}_{\mathrm{Treatment\ I}}$ indicates intake of the control solution by workers, $\mathrm{Abs}_{\mathrm{Treatment\ II}}$ indicates intake of the conidial solution by RIFA. Thus, PREF values represent the degree of preference of RIFA for conidia; values less than 1 represent a preference for conidia and larger than 1 values represent aversion.

2.5. Feeding choice between paired congeneric fungi

The method to test the choice of RIFA between congeneric fungi was modified from that described in the feeding preference experiment. Briefly, four pieces (1 × 1 cm) of filter paper were placed equidistantly at the bottom of each plastic box. In Treatment I, two diagonal pieces were treated with the least pathogenic fungi with 40 µl of 5×10^7

conidia/ml suspension including 2 mg/ml of brilliant blue suspended in 0.01% Tween-80 solution, while two other pieces were treated with 40 µl of a colourless 5×10^7 conidia/ml suspension of the relatively higher pathogenic congeneric fungi. Opposing control experiments were set up to avoid the influence of brilliant blue. Each treatment was repeated 10 times within a 24-h period. Thereafter, RIFAs were homogenised and centrifuged, and the absorbance of each supernatant was measured as mentioned above. The choice index (CI) between congeneric microbial solutions was calculated from the following equation:

$$CI = (Abs_{Treatment\ I} - Abs_{Treatment\ II})/(Abs_{Treatment\ I} + Abs_{Treatment\ II})$$

In this equation, $Abs_{Treatment\ I}$ indicates the intake of the least pathogenic fungi and $Abs_{Treatment\ II}$ indicates the intake of congeneric fungi with higher virulence. Thus, CI values are limited between 1 and –1, and positive values indicate a preference for lower pathogenic fungi and negative values indicate aversion.

2.6. Statistical analysis

All data were analysed with SPSS 13.0 software. For mortality bioassays, the median lethal concentration (LC_{50}) and median lethal time (LT_{50}) were examined using Probit regression analysis. Proportional mortality data were compared by one-way analysis of variance (ANOVA). An independent samples *t*-test was used to analyse feeding preference of RIFA. Before performing ANOVA and the *t*-test, data were tested for normality using the Shapiro–Wilk test. Levene's test was used to analyse homogeneity of variance after log transformation for both variables. The relationship between pathogenicity and feeding preference was tested by the determination coefficient. Food choice between congeneric fungi was analysed with an independent samples *t*-test.

3. Results

3.1. Pathogenicity bioassay

Treatment with a conidial suspension of each isolate (Table 1) resulted in mortalities that varied according to isolate (Table 2). At a concentration of 1×10^8 spores/ml, Bb04, Ma01 and M09 isolates caused over 90% mortality. Moreover, M09 caused complete mortality at concentrations of 1×10^7 and 1×10^8 spores/ml. In contrast, IFCF-L, Bb02 and SM076 isolates caused lower mortality compared to other isolates at these concentrations. The LT_{50} of isolates at 1×10^7 and 1×10^8 conidia/ml is presented in Table 2 and the LC_{50} in Table 3. When compared within the same genera, the orders of pathogenicity were IFCF-H > IFCF-L in *Isaria*, Bb04 > Bb02 in *Beauveria* and M09 > Ma01 > SM076 in *Metarhizium*.

3.2. Feeding preference

The results of RIFA's feeding preference are shown in Table 4. RIFA consumed significantly less Bb04 than the control ($t = -3.01$, $df = 18$, $P = 0.008$). RIFA did not show a significant taste response to the conidia of six remaining isolates. The PREF value of all *B. bassiana* isolates was less than one ($PREF_{Bb02} = 0.3$, $PREF_{Bb04} = 0.09$), in which $PREF_{Bb04}$ was the lowest. In contrast, the PREF value of *M. anisopliae* isolates was higher than one ($PREF_{Ma01} = 2.02$, $PREF_{SM076} = 2.05$, $PREF_{M09} = 1.14$), in which $PREF_{SM076}$ was the highest among all the isolates.

Table 2. Median lethal times (LT_{50}) of isolates belonging to *Isaria*, *Beauveria* and *Metarhizium* at concentrations of 1×10^7 and 1×10^8 conidia/ml against *S. invicta* workers. All the data calculated were at 10 days *post*-inoculation.

		LT_{50} values, days (95% fiducial limits)		% mortality at day 10 (±SE)	
Fungal species	Isolates	1×10^7 conidia/ml	1×10^8 conidia/ml	1×10^7 conidia/ml	1×10^8 conidia/ml
I. fumosorosea	IFCF-H	7.3 (6.5–8.5)	3.4 (2.6–4.1)	65.5 ± 12.5b	87.8 ± 5.6d
	IFCF-L	162.6 (46.1–1.5E + 11)	42.5 (23.5–151.1)	11.7 ± 1.0e	17.5 ± 0.8bc
B. bassiana	Bb02	17.6 (12.8–33.8)	7.3 (6.6–8.2)	31 ± 6.1cd	66.7 ± 8.3c
	Bb04	4.0 (3.7–4.3)	2.8 (2.5–3.2)	80.8 ± 3.1ab	99.2 ± 0.8a
M. anisopliae	Ma01	7.5 (6.9–8.1)	3.8 (3.4–4.2)	55.8 ± 11.3b	88.8 ± 0.6c
	SM076	8.5 (7.7–9.5)	7.3 (6.8–7.8)	60 ± 0.7bc	70.8 ± 0.8bc
	M09	4.1 (3.9–4.3)	2.7 (2.6–2.9)	100 ± 0a	100 ± 0a
Control			17.5 (14.7–22.7)		

Means followed by the different letter are significantly different within a column ($P = 0.05$).

3.3. *Relationship between pathogenicity of fungi and feeding preference of* S. invicta

There was no correlation between pathogenicity (LC_{50}) and corresponding feeding preference (determination coefficient $R^2 = 0.013$; Figure 1). However, there seemed to be a negative trend between LC_{50} and corresponding feeding preference within congeneric isolates. Within congeneric isolates, the isolate of lower pathogenicity appeared to be more acceptable to RIFA (Figure 2).

3.4. *Feeding choice between congeneric fungi*

The results of feeding choice between paired conidial solutions of congeneric fungi are shown in Table 5. CI values in all the paired experiments were positive indicating that RIFAs prefer feeding solutions with conidia of the relatively lower pathogenic isolate. In *I. fumosorosea*, the intake of solution containing conidia of IFCF-H was

Table 3. Median lethal concentration (LC_{50}) values of isolates belonging to *Isaria*, *Beauveria* and *Metarhizium* against *S. invicta* workers on the tenth day *post*-inoculation.

Fungal species	Isolates	LC_{50}[a]	Limits (conidia/ml)	Regression equation[b]	χ^2	P[c]
I. fumosorosea	IFCF-H	1.20×10^7	$5.8 \times 10^6 - 2 \times 10^7$	m = 5.44c–5.14	0.30	0.58
	IFCF-L	1.56×10^{10}	–	m = 1.06c–1.33	0.620	0.43
B. bassiana	Bb02	4.23×10^7	$2.51 \times 10^7 - 6.59 \times 10^7$	m = 5.11c–5.03	0.275	0.60
	Bb04	3.04×10^6	$1.53 \times 10^6 - 4.86 \times 10^6$	m = 6.65c–6.15	0.004	0.95
M. anisopliae	Ma01	6.13×10^6	$9.08 \times 10^5 - 1.50 \times 10^7$	m = 4.61c–4.01	5.948	0.051
	SM076	2.90×10^7	$4.09 \times 10^6 - 7.49 \times 10^7$	m = 3.36c–3.17	1.44	0.23
	M09	9.90×10^5	$4.03 \times 10^5 - 1.72 \times 10^6$	m = 4.05c–3.86	1.12	0.29

Note: LT_{50} until the tenth day of the control group are shown in Table 2.
[a]Concentrations are number of conidia per millilitre.
[b]m, probit transformed mortality; c, concentration.
[c]Homogeneity for the fit was accepted if $P > 0.05$ for the χ^2 test ($df = 2$).

Table 4. Feeding preferences of *S. invicta* workers for *Isaria*, *Beauveria* and *Metarhizium* isolates ($p < 0.05$).

Fungal species	Isolates	Abs treatment I	Abs treatment II	*P* value	PREF	Odour effect
I. fumosorosea	IFCF-H	0.04 ± 0.01	0.05 ± 0.08	0.354	0.75	Neutral
	IFCF-L	0.19 ± 0.03	0.19 ± 0.04	0.933	1.02	Neutral
B. bassiana	Bb02	0.04 ± 0.01	0.13 ± 0.05	0.297	0.30	Neutral
	Bb04	0.02 ± 0.01	0.23 ± 0.07	0.008[a]	0.09	Repellent
M. anisopliae	Ma01	0.13 ± 0.06	0.07 ± 0.15	0.308	2.02	Neutral
	SM076	0.12 ± 0.02	0.06 ± 0.03	0.06	2.05	Neutral
	M09	0.04 ± 0.01	0.04 ± 0.01	0.77	1.14	Neutral

[a]Represents significant difference of absorbance (mean ± SE) between Treatment I (pathogenic fungi) and Treatment II (control solution) (independent samples *t*-test, $p = 0.05$).

significantly less than IFCF-L ($t = 2.34$, $df = 18$, $P = 0.031$), suggesting that IFCF-H suppressed the feeding activity of RIFA. A similar result was observed for *Beauveria.* Bb02 intake was barely significantly less than Bb04 ($t = 2.16$, $df = 18$, $P = 0.044$). SM076 was barely more preferred than Ma01 ($t = 2.14$, $df = 18$, $P = 0.046$) or M09 ($t = 2.12$, $df = 18$, $P = 0.049$). There was no significant choice tendency between Ma01 and M09 ($t = -0.142$, $df = 18$, $P = 0.888$).

4. Discussion

IFCF-H, Bb04, Ma01 and M09 showed higher virulence with mortalities in RIFA ranging from 87.8% to 100% at a concentration of 1×10^8 conidia/ml until the 10th day. Also related, these four isolates showed lower estimated LT_{50} values ranging from 2.7 to 3.8 days at the same conidial concentration. These results indicate the great potential of these isolates to manage RIFA in the field.

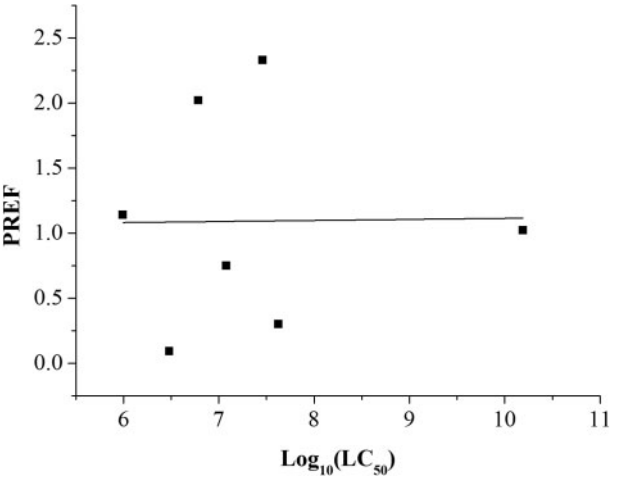

Figure 1. Relationship between the pathogenicity (log transformed of LC_{50}) of fungal isolates and feeding preference (PREF) of *S. invicta*.

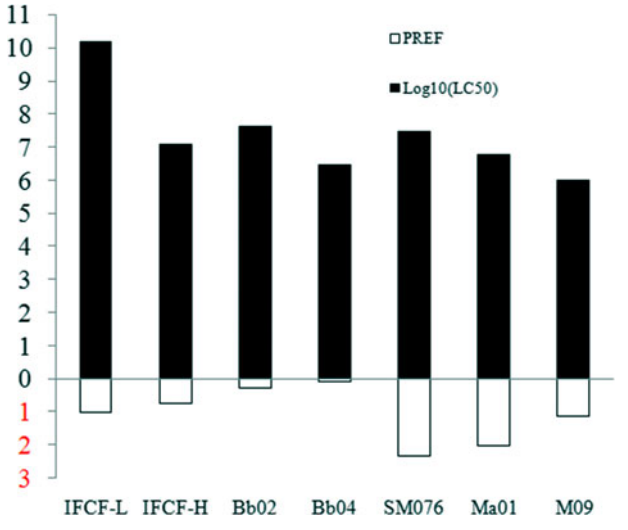

Figure 2. (Colour online) Pathogenicity (log transformed of LC_{50}, ■) of fungal isolates and corresponding feeding preference (PREF, □) of *S. invicta*.

In social insects, infected individuals are either treated with intensive grooming, avoided or even attacked, by their nestmates. This can result in the failure of inducing an epizootic in the nest (Baracchi, Fadda, & Turillazzi, 2012; Bos, Lefevre, Jensen, & D'Ettorre, 2012; Yanagawa & Shimizu, 2007). Therefore, for any entomopathogenic fungus for the control of RIFA, pathogenic and non-deterrent isolates are preferred. In our study, RIFA showed differences in feeding preferences for fungi belonging to different genera. The PREF values were all larger than 1 in *Metarhizium* but less than 1 in *Beauveria*. This suggests that RIFA prefers *Metarhizium* over *Beauveria*. In addition, RIFA showed no significant taste response to *Isaria*. Therefore, the feeding preference of RIFA for these fungal genera appears to be in the order of *Metarhizium* > *Isaria* > *Beauveria*. In previous studies, IFCF-H caused higher mortality and a lower electroantennogram responses of

Table 5. Feeding choices of *S. invicta* between paired congeneric isolates belonging to *I. fumosorosea*, *B. bassiana* and *M. anisopliae* ($p < 0.05$).

Fungal species	Pair	Abs treatment I	Abs treatment II	*P* value	Choice index (CI)
I. fumosorosea	IFCF-L vs. IFCF-H	0.29 ± 0.04	0.17 ± 0.04	0.031*	0.26
B. bassiana	Bb02 vs. Bb04	0.15 ± 0.018	0.10 ± 0.015	0.044*	0.20
M. anisopliae	SM076 vs. M09	0.32 ± 0.03	0.21 ± 0.04	0.049*	0.21
	SM076 vs. Ma01	0.34 ± 0.057	0.20 ± 0.03	0.046*	0.26
	Ma01 vs. M09	0.34 ± 0.07	0.33 ± 0.04	0.888	0.01

*Represents significant difference in food intake indicated by absorbance (mean ± SE) between Treatment I (lower pathogenic isolate) and Treatment II (congeneric isolate with relatively higher pathogenicity), (independent samples *t*-test, $p = 0.05$).

Coptotermes formosanus workers than *Beauveria* and *Metarhizium* (Hussain et al., 2010; Hussain, Tian, He, & Lei, 2010). In our study, IFCF-H caused high mortality and no feeding response to RIFA, suggesting that IFCF-H research for its application to control RIFA or other subterranean termites.

There was a negative relationship between fungal pathogenicity and feeding preference of RIFA (Figure 2). Thus, we proved this relationship with the feeding choice experiment among congeneric fungi. Our result shows that RIFA prefer the fungal isolate of lower pathogenicity. This is consistent with the research of Mburu et al. (2009), who reported that fungi can inflict the insect and that virulent isolates are more likely to be recognised from some distance and avoided. There are two potential explanations for this phenomenon. First, differences in physiological characteristics and enzyme production among fungal isolates may be responsible for the observed variation in their virulence against RIFA. These differences among fungal isolates result in alterations of microbial VOCs of the conidia which RIFA can detect and discriminate. A second possible mechanism is that RIFA are capable of detecting the mycotoxins produced by a fungus. However, Cheraghi, Habibpour, and Mossadegh (2013) reported that *M. anisopliae* DEMI001, despite being highly pathogenic, showed no repellency to termites. Rather, it caused high mortality of termites consuming the lethal dose of bait containing its conidia in the field. Thus, the impacts of VOSs and mycotoxins on the behaviour of RIFA should be studied, and more isolates are needed to prove whether this phenomenon is general in nature.

Based on the results of pathogenicity, feeding preference and feeding choice bioassays in this study, we conclude that pathogenicity of congeneric fungi would affect the feeding preference of RIFA. IFCF-H, Bb04, Ma01 and M09 were pathogenic to RIFA. Therefore, these four isolates need further research for their potential to manage RIFA as a powder formulation in the field. Moreover, although SM076 showed less virulence against RIFA, its conidia are readily accepted and consumed. Targeted use of sublethal doses of insecticide together with entomopathogenic fungi will accelerate the performance of fungi (Ramakrishnan, Suiter, Nakatsu, Humber, & Bennett, 1999). Thus, we suggest that low and moderately pathogenic entomopathogenic fungi are added more readily and more effectively when formulated as baits to control RIFA in the field. In contrast, Bb04 was toxic and deterred RIFA the most. Further studies are needed to assess its potential application in the control of agricultural pests.

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